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Specific Induction of the 93D Puff in *Drosophila melanogaster* by a Homogenate of Heat Shocked Larval Salivary Glands

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The effect of a homogenate of heat-shocked (60 min at 37°C) larval salivary glands (HSGH) of *D. melanogaster* on the activity of the different heat shock puff loci in fresh salivary glands has been examined by ^{3}H-uridine autoradiography. A 45 min incubation of fresh glands in HSGH results in induction of only the 93D puff without a concurrent induction of the other eight heat shock puff loci. Other chromosomal and nucleolar RNA synthesis is not affected. A 20 min heat shock at 37°C to glands preincubated in HSGH causes a typical induction of all the other eight heat shock puff loci; the 93D puff activity in these doubly treated glands is slightly less than in only HSGH incubated glands. These observations are discussed in relation to regulation of the 93D puff activity.

The temperature or heat shock (TS) induced alterations in gene activity in *Drosophila* have been widely studied in recent years and these studies have provided valuable information about the regulation of gene activity at transcriptional and translational levels (see review by Ashburner and Bonner[1]). We have earlier shown[2] that of the nine specific heat shock inducible puff loci in polytene chromosomes of *Drosophila melanogaster*[3], the response of the puff 93D is independent of the other eight TS puffs and moreover, an induced change in the activity pattern of the 93D puff by benzamide modifies the entire TS response[4]. In this report, we present our observations on the activity of the TS puff sites in salivary glands incubated in a homogenate of larval salivary glands previously exposed to heat shock. These observations further show that the regulation of the 93D puff activity in *D. melanogaster* is independent of the other TS puffs.

Materials and Methods

These studies were done with salivary glands dissected out from healthy late third instar larvae of the *D. melanogaster* (Oregon R$^+$). The medium used for dissecting and incubating the glands contained only the inorganic salt constituents of Poels'[5] tissue culture medium.

For obtaining a homogenate of heat shocked salivary glands, thirty pairs of freshly excised salivary glands from late third instar larvae were kept in 10 μl of the Poels' inorganic salt solution in a cavity slide and sealed with a coverglass. The sealed cavity slide was then kept at 37°C for 60 min following which the cavity slide was brought to room temperature (24°C), the coverglass was removed and the glands were crushed and homogenized in the 10 μl solution in the cavity with a small glass rod in as fine a homogenate as possible. A microscopic examination revealed that there were no intact polytene chromosomes or nuclei in the homogenate. Similarly, thirty pairs of glands were incubated in 10 μl of the salt solution at 24° ± 1°C for 60 min and then these glands were also homogenized as above. These two homogenates would be referred to as "Heat shocked gland homogenate" (HSGH) and "Control gland homogenate" (CGH), respectively. Freshly dissected sister salivary glands were separated and incubated at 24°C for 45 min either in the HSGH or in CGH. After 45 min, the glands were washed with fresh Poels' salt solution and then labelled with ^{3}H-uridine (activity 500 μCi/ml; sp. act. 10.9 mCi/mM, BARC, Trombay) for 10 min at 24°C. The labelled glands were then fixed, squashed and autoradiographed with Ilford L4 nuclear emulsion in the usual manner.

In another set, freshly dissected salivary glands were incubated in the HSGH for 45 min at 24°C as above and were then subjected to heat shock (37°C) for 30 min. Following these two treatments, the glands were labelled with ^{3}H-uridine as above for 10 min at 24°C. The labelled glands were then fixed, squashed and processed for autoradiography.

The autoradiographic preparations in each case were scored for the degree of labelling (in terms of silver grain counts) of two chromosome segments (1A to 3C on X chromosomes and 60A to 63B on 3L), the nucleolus, the chromocenter (β-heterochromatin) and the six major TS puff sites (63BC and 67B on 3L, 87A, 87C, 93D and 95D on 3R) as described earlier[4].

Results

The data on the ^{3}H-uridine labelling (mean silver grain counts) on the segments of X chromosome and

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3L, on the nucleolus and on the β- heterochromatin of the chromocenter in polytene nuclei after the three treatments are presented in Table 1. The rate of RNA synthesis is somewhat higher in salivary glands incubated in HSGH than in the sister glands incubated in CGH, since the mean silver grain counts on all the four regions are slightly higher in the former preparations (Table 1). However, a heat shock to glands preincubated in HSGH, causes a severe inhibition of ^{3}H-uridine incorporation in these regions; the inhibition is more in chromosomal regions than in the nucleolus while the incorporation in β-heterochromatin is nearly completely inhibited (see Table 1 and Fig. 1).

The data on the activity of the six TS puffs after the treatments are presented in Table 2. In this table we have also included data on the labelling of these six TS puffs after a routine 20 min heat shock at 37°C (data taken from Lakhotia and Mukherjee[4]) for comparison with results of the present treatments. It is seen that while 45 min incubation in CGH does not induce any of the TS puff sites, the HSGH incubation causes the 93D puff to be highly activated without a concurrent induction of any of the other TS puffs (see Table 2 and Fig. 1). It is to be noted that the induced activity of the 93D puff varies in different nuclei in HSGH incubated glands since the grain counts on the 93D puff in the nuclei included in the data in Table 1 varied from 62 to 166. Besides, in some nuclei the labelling of the 93D puff was too high to be counted. Heat shock to HSGH preincubated glands results in activation of all the TS puff loci and the level of ^{3}H-uridine incorporation in all these puffs, other than 93D, is nearly similar to that seen after a routine heat shock (compare row D with A in Table 2). The mean number of silver grains on the 93D puff in glands exposed to heat shock after HSGH incubation is more than that seen after a routine heat shock but less than in glands only incubated in HSGH (see Table 2). These mean grain counts are significantly different ($P < 0.05$) from each other. Although we have not specifically looked for the activity of other non-TS puff sites in the CGH and HSGH incubated glands, we did not notice any striking induction of activity of any other puff site in either cases (see Fig. 1a,b).

Discussion

Using an *in vitro* system developed by Compton and Bonner[6], Compton and McCarthy[7] had reported that when fresh salivary glands of *D. melanogaster* larvae are disrupted in cytoplasm from heat-shocked *Drosophila* Kc cells in culture, the TS puff loci are induced in the polytene nuclei. However, it was noted[7] that the 93D puff does not respond to this *in vitro* system. In this context our present results are significant. Unlike the results of Compton and McCarthy[7], we have observed that incubation of intact larval salivary glands in HSGH results only in the induction of 93D puff while the other TS puff loci do not respond. There are two important differences in the experimental procedures used by us and by earlier workers[7]. Firstly, while we have used a homogenate of entire larval salivary glands as the incubation medium,

Table 1—^{3}H-Uridine Incorporation in Chromosome Segments, Chromocentre and Nucleolus after the Different Treatments

Treatment	Mean (± SE) No. silver grains on			
	X segment (1A-3C)	3L segment (60A-63B)	Chromo-centre	Nucleo-lus*
CGH incubation (45 min, 24°C)	98.73 ±4.47 (26)**	89.19 ±3.14 (27)	48.27 ±2.43 (33)	27.08 ±0.68 (36)
HSGH incubation (45 min, 24°C)	110.25 ±2.26 (31)	94.12 ±3.68 (42)	60.26 ±1.94 (34)	30.45 ±0.61 (44)
HSGH incubation (45 min, 24°C) followed by TS (37°C, 20 min)	33.36 ±4.18 (14)	33.12 ±2.13 (34)	4.00 ±0.46 (25)	15.44 ±0.77 (43)

*For nucleolar grain density, grains were counted over three separate regions (each 25 μm^2) of the nucleolus in a nucleus and the average of the three values was taken as the nucleolar labelling.

**Figures in parentheses indicate the total number of nuclei examined in the respective case.

Table 2—^{3}H-Uridine Incorporation in Major Heat Shock Puff Loci after Different Treatments

Treatment	Mean number of grains (± SE) on different TS puff sites					
	93D	95D	87A	87C	63BC	67B
A. Normal Heat Shock * (37°C, 20 min)	76.12 ±2.50 (42)**	22.72 ±3.80 (39)	65.32 ±2.50 (38)	64.98 ±3.10 (38)	40.09 ±1.70 (41)	54.08 ±2.13 (40)
B CGH incubation (45 min, 24°C)	31.70 ±1.07 (37)	12.00 ±0.62 (37)	8.67 ±0.56 (31)	5.68 ±0.56 (31)	19.66 ±0.56 (28)	11.76 ±0.69 (21)
C HSGH incubation (45 min, 24°C)	107.35 ±4.25 (46)	14.73 ±0.89 (45)	9.54 ±1.68 (37)	6.73 ±0.45 (37)	15.86 ±0.75 (44)	15.56 ±0.86 (32)
D HSGH incubation (45 min, 24°C) followed by TS (20 min, 37°C)	94.46 ±4.14 (48)	26.02 ±1.22 (48)	69.00 ±3.43 (47)	72.70 ±3.29 (47)	44.23 ±1.54 (48)	49.89 ±2.88 (48)

*Data from Lakhotia and Mukherjee[4].

**Figures in parentheses indicate the number of nuclei examined in each case.

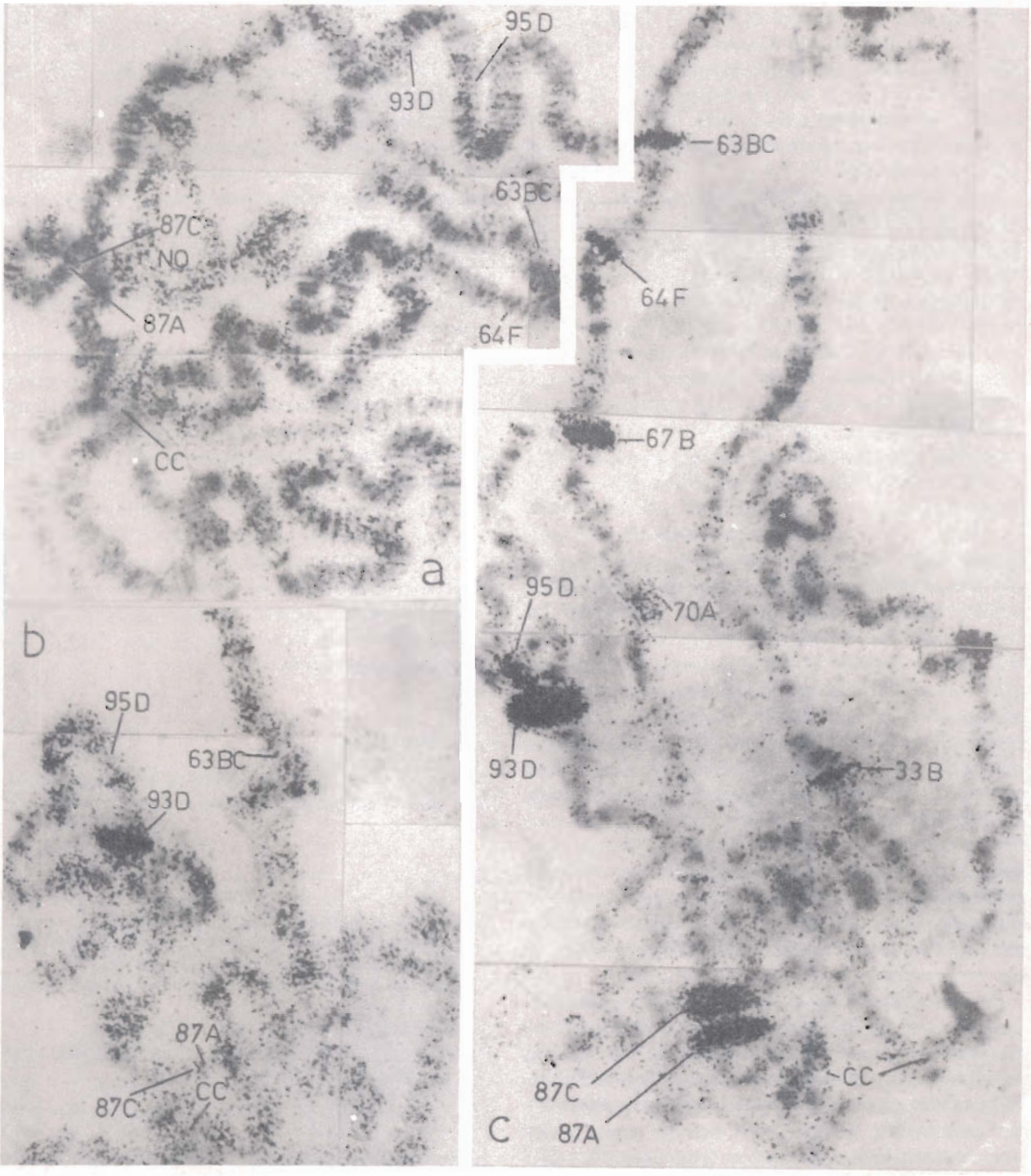

Fig. 1—Autoradiographs of ^{3}H-uridine labelled polytene nuclei of *D. melanogaster* from CGH incubated (a), HSGH incubated (b), and HSGH pre-incubated followed by a heat shock treated (c) glands. The cytological location of some or all of the heat shock puff sites in different nuclei is indicated. **CC** = chromocentre; NO = nucleolus. (Magnification = × 1200)

they[7] had taken only the cytoplasmic part of an embryonic cell line. Secondly, while we have incubated intact salivary glands in the homogenate, Compton and McCarthy[7] disrupted the salivary gland cells prior to incubation. It appears to us that the opposite results with regard to the induction of TS puffs obtained by us and by Compton and McCarthy[7], are related to the above two differences in the experimental conditions and are not due to the use of a homogenate of embryonic or salivary gland cells as such, since the overall heat shock response is similar in embryonic and larval salivary gland cells[8-10].

Our earlier observations[2] on the independent response of the 93D puff to TS together with the results of Compton and McCarthy[7] and the present data, clearly show that the regulatory pathways involved in the induction of the 93D puff are different from those for the other TS puffs. There is another feature of the induced activity of 93D puff which is relevant to the present observations. Unlike the other TS puffs, the 93D puff regresses very quickly after the withdrawal of the inducing agent. Thus we have seen (unpublished data) that when heat shocked salivary glands are kept at 24°C, within 10-20 min the 93D puff regresses nearly completely and is also much less active in RNA synthesis; on the other hand, the other TS puffs still remain nearly as active as they are immediately after the heat shock. Similarly, the benzamide induced 93D activity regresses soon after the withdrawal of benzamide[4]. This means that for the maintenance of the induced activity of the 93D locus, the inducing agent has to be constantly present. It would, therefore, appear that in our HSGH, the factor/s directly responsible for induction of the 93D puff, generated due to the heat shock, are present and they are able to activate the 93D puff in fresh glands. Apparently, these specific factor/s are absent in Compton and McCarthy's[7] *in vitro* system. Since in their *in vitro* system the nuclear components of the heat shocked cells are not included in the incubation medium, while they are included in our HSGH, it appears that the factor/s directly responsible for the 93D puff induction are intranuclearly located.

The non-inducibility of the other puffs under our conditions of HSGH incubation may be due either to the inactivation in the homogenate of the factor/s essential for the induction of these puffs (other than 93D) or to the inability of these factor/s to interact with the genome in fresh glands due to their intact nucleocytoplasmic organization. This aspect is being further studied.

The selective induction of the 93D puff by HSGH incubation is comparable to the effect of short *in vitro* benzamide (BM) treatment since with the latter treatment also[4,11], only the 93D puff is induced without a concurrent induction of the other TS puffs. However, these two pretreatments modify the subsequent TS response in different ways. When BM pretreated glands are exposed to heat shock, all the TS puffs are induced to a lesser extent than after heat shock to non-BM treated glands and moreover, in the doubly treated glands the 87C puff is much less active than 87A puff[4]. However, the present data show that heat shock to HSGH preincubated glands results in a near normal induction of all TS puffs (except the 93D puff, see below). These differences in the responsiveness of BM- or HSGH-incubated glands to subsequent heat shock may be related to the divergent effects of the BM and HSGH treatments on chromosomal RNA synthesis: BM drastically inhibits chromosomal RNA synthesis[4,11], but as seen here HSGH incubation does not have any inhibitory effect on the chromosomal or nuclear RNA synthesis.

The lower mean grain counts on the 93D puff in doubly treated glands (TS after HSGH incubation) than in only HSGH incubated glands (Table 2) shows that the second treatment (TS) actually causes some inhibition of the earlier induced activity of the 93D locus rather than additively inducing its activity. In our earlier studies[4] on BM and TS combination treatments also, a similar pattern of response of the 93D locus was noted. The mechanism and implications of inhibition of the 93D activity by the second inducing treatment are not known at present. Further analysis of these aspects would help in a better understanding of the organization and significance of the activity of the 93D locus.

Acknowledgements

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